Symmetric Division of Cancer Stem Cells – a Key Mechanism in Tumor Growth that should be Targeted in Future Therapeutic Approaches

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As cancer stem cells (SCs) drive tumor growth, it is only through the elimination of those cancer SCs that a pharmacologic cure can be attained. To study ways to develop drugs that target cancer SC, we investigated changes in cellular mechanisms and kinetics that occur in SC populations during colorectal cancer (CRC) development. We used computer modeling to determine which changes could give rise to exponential increases in both SC and non-SC populations in CRC. Our results show that the only mechanism that can explain how these subpopulations increase exponentially in CRC development involves an increase in symmetric SC cell division. This finding suggests that any systemic therapies designed to effectively treat CRC and other cancers must act to control or eliminate symmetrical cancer SC division in tumors, while minimally affecting normal SC division in non-tumor tissues.

Curing cancer in its advanced stages is difficult. This may be due to the current systemic treatment approach in which chemotherapeutic agents are designed to kill rapidly proliferating cells, which make up the bulk of tumors. Among the many reasons why chemotherapy does not kill all malignant cells in a cancer, three seem particularly salient: (1) some cells in tumors are insensitive to chemotherapy through mechanisms such as multidrug resistance; (2) Chemotherapeutic agents are not specific enough and kill normal cells as well, and this toxicity limits the maximal tolerable dose; (3) some proliferative cells in tumors are not proliferating. However, new insights towards more effective treatment approaches are emerging from a new tenet in oncology – that cancers arise from adult stem cells (SCs) and that cancer SCs drive tumor growth.[1–3] Indeed, SCs are known to be quiescent or only slowly proliferating and inherently possess mechanisms for drug resistance such as multidrug resistance. As a result, cancer SCs are becoming recognized as a necessary target for effective anticancer therapy.[1,4]

In this view, the task now is to control or eliminate cancer SC populations. To this end, we will need to identify mechanisms by which cancer SC drive tumor growth and to target those mechanisms. Identifying such mechanisms will, necessarily, require an understanding of mechanisms that give rise to the unique properties of SC in normal tissues (**Table 1**) and how these mechanisms become altered when a normal SC becomes a cancer SC. Accordingly, we focused on tumor growth in colonic tissue and developed a mathematical model to explore likely cellular mechanisms of that tumor growth.

An approach that we thought was both novel and key was to study the changes in cell populations in tissues that are characteristic of cancer development. We thus designed a mathematical model to study the dynamics of cell populations (rather than what happens to individual cells) and how changes in these dynamics might affect the various cell populations in colonic tissues during colorectal cancer (CRC) development. As part of our modeling, we used information from recent studies regarding cell populations in colonic tumors, studies that show that within each CRC a small but significant proportion (0.25–2.5%) of tumor cells are cancer SCs.[5,6] As CRCs contain an exponentially increased number of neoplastic cells (10^8–10^{13}), the size of the SC population must also have increased exponentially. If SCs in tumors continue to be $\sim$1% of the total cell population of the tumor, then the increase must have been from about 20 SCs in the normal crypt to 10^6–10^{11} cancer SCs in a colon cancer. The purpose of our modeling study was to determine what changes could give rise to such exponential increases in SC and non-SC populations. We approached this with the objective of identifying key mechanisms in tumor growth that represent targets for new, potentially curative pharmacological approaches to cancer treatment.

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Published online 25 April 2007. doi:10.1038/sj.clpt.6100202

Table 1 Properties of SC, IC, and NC populations

Population property	SC population	IC population	NC population
Self-renewal	SC population is able to independently maintain itself	Maintenance of the IC population depends on the SC population	Maintenance of the NC population depends on the SC and IC populations
Capacity for multi-lineage differentiation	SCs are undifferentiated cells with the capacity for producing a variety of differentiated cell types	ICs are committed to produce specific differentiated cell types	NCs are terminally differentiated, apoptotic or terminally injured by cytotoxic agents (radiation, chemotherapeutics, etc)
Proliferative capacity	Proliferative cells having the capacity for cell division over the lifetime of the host organism	Proliferative cells having the capacity for cell division over the short-term	Non-proliferative cells having zero capacity for cell division
Fraction of overall cell population	Small (~1%)	Large	Large
Proliferation status	SCs are quiescent or slowly proliferating	ICs are rapidly proliferating	NCs are nonproliferating
Capacity for tissue renewal	SCs have the capacity for ongoing tissue renewal and tissue generation during development and regeneration after injury	ICs have lost the capacity for ongoing tissue renewal and tissue generation during development and regeneration after injury	NCs have zero capacity for ongoing tissue renewal and tissue generation during development and regeneration after injury

IC, intermediate cell; NC, nonproliferative cell; SC, stem cell.

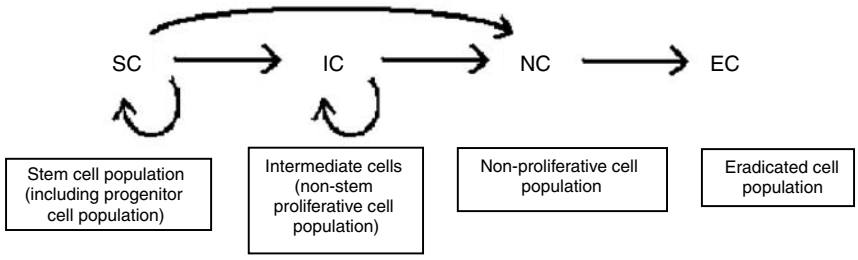

Figure 1 Model for the proliferative kinetics of normal and malignant tissues. There are four types of cell populations in our model: SC, IC, NC, and eradicated cell. SC and IC represent proliferative cells. Although the SC population is self-renewing, the IC population is normally not self-renewing and maintenance of the IC population requires production of new IC through SC division. NCs are produced from SCs and ICs and production of NCs is not a product of cell division. NCs do not divide, but can be lost from the system through the process of eradication, *i.e.*, eradicated cell production.

RESULTS

We structured a biologically relevant model based on biological evidence. There are four types of cell populations in our model: SC, IC, NC, and eradicated cell. Dynamics of these four types of cell populations are described by mechanisms and rate reactions shown in **Figures 1** and **2** and their properties are given in **Table 1**. SC and IC represent proliferative cells. As shown in **Figure 2a**, SC divide either symmetrically to form two SC (Rxn 1), two IC (Rxn 3), or asymmetrically to form an SC and an IC (Rxn 2). SC can also become NC (Rxn 4). The SC population is self-renewing through symmetric (Rxn 1) and asymmetric (Rxn 2) SC division. IC divides symmetrically to form two IC (Rxn 5) or become NC (Rxn 6). The IC population is normally not self-renewing and maintenance of the IC population requires production of new IC through SC division (Rxns 2 and 3). NCs are produced from SC (Rxn 4) and IC (Rxn 6), and production of NC is not a product of cell division. NCs do not divide, but can be lost from the system through the process of eradication, *i.e.*, eradicated cell production (Rxn 7).

Computer modeling of normal colonic crypt populations (**Figure 3a**) generated output showing a constant number of SC, intermediate cell (IC), and non-proliferative cell (NC) with time, which is consistent with a steady-state condition. Increasing ($20\times$) the rate of asymmetric SC division (Rxn 2) produced an increase in IC and NC, but not in SC (**Figure 3b**). In this case, the number of IC and NC asymptotically approached a maximum in the range of 10,000–40,000 cells at 4 years (~35,000 h). In contrast, increasing the rate of symmetric IC division (Rxn 5) and of symmetric SC division (Rxn 1) produced an exponential increase in total cell number over time (**Figure 3c** and **d**). **Figure 3c** shows that increasing symmetric IC division (by as little as $1.2\times$) produces a rapid exponential increase in IC and NC number ($>10^9$ cells by ~0.25 year or ~2,000 h), but did not increase SC number. In contrast, **Figure 3d** shows that increasing symmetric SC division ($20\times$) produces a slow exponential increase (total cells $>10^9$) over 4 years (~35,000 h) in IC, NC, and SC. In this case, SC ($\sim10^8$) represents a small subpopulation (~3%) of the total cell population. Decreasing the type of symmetric SC division that produces two ICs (Rxn 3) had minimal effect on SC or IC number, probably because the slight increase in SC number due to decreased SC loss is offset by a decrease in IC production (data not shown). Decreasing the rate of production of NCs from SC (Rxn 4)

had only a modest effect on increasing SC and IC number (an increase of less than 3,000 cells over 30,000 h – data not shown).

DISCUSSION

Results from our modeling not only provide insight into cell kinetic mechanisms that drive tumor growth, but also have implications for developing new approaches toward cancer treatment. Our previous studies[7–9] provided evidence suggesting that SCs are overproduced in the initiation and progression steps of human colon tumorigenesis. Two more recent studies[5,6] provided important information that cancer SCs are contained in human CRCs and that they represent about 1% of the total number of cells within the tumor. These results are consistent with studies showing that various cancer types, including malignancies of the breast,[10] pancreas,[11] head and neck,[12] neurologic,[13–15] and hematologic system,[16–21] also contain a small fraction (~1%) of cancer SCs. As cancers, including CRC, at the time of diagnosis contain an exponentially increased number of tumor cells (10^8–10^{13}) relative to normal tissues, the cancer SC population within tumors must also have increased exponentially (10^6–10^{11} cells).

As cancer SCs drive tumor growth, it is only through the elimination of those cancer SCs that a cancer cure can be attained. It follows, then, that the most productive research approach to develop effective anticancer therapies is to focus on changes in cellular mechanisms and kinetics that occur in SC populations during cancer development.

The specific question we asked was – which reaction in cell population dynamics, when changed, can account for the biologic characteristics of CRC development? This was done by seeing if model output fit with three of these characteristics: (i) CRC development leads to an exponential increase in both SC and non-SC, (ii) CRC development involves maintenance of a small but relatively constant ratio of SC/non-SC number, and (iii) CRC development takes a considerably long time (2–10 years). In considering the seven reactions shown in **Figures 1** and **2**, changes in only one of them, reaction 1, explained the above biological characteristics of CRC development. The output of our model for that perturbation showed (1) an exponential increase ($>10^8$ cells) in both SC and non-SC populations (IC and NC), (2) a relatively constant SC/total cell population ratio (~3%), and (3) a relatively long time period (~4 years) before the exponential increase in cell number was reached. Model output from perturbations of any of the other six rate constants shown in **Figure 2** was unable to account for these three biological characteristics. In this view, when the rate of symmetrical SC division increases, then SC population size increases which, in turn, increases IC population size through SC asymmetric cell division. Both increased SC and IC population sizes will lead to an increase in NC population size. Consequently, increased symmetric SC cell division would result in exponential growth of all three populations, SC, IC, and NC.

So what are the clinical implications of our results that indicate increased SC division is the driving force for cancer growth? For one, there needs to be an effort to develop drugs that specifically target symmetric SC division of cancer SCs. This is a challenging task because it will be necessary to distinguish cell pathways involved in regulating the two different processes involved in SC self-renewal – symmetric versus asymmetric SC division. This task will have to address the fact that any potential drug that has a major inhibitory effect on asymmetric SC division will only inhibit production of IC, not reduce cancer SC numbers. Nonspecifically inhibiting asymmetric SC division (a mechanism sometimes misconstrued to be the only process involved in SC self-renewal) would carry potential toxicity to normal tissue because our modeling indicates that this type of SC division is a main mechanism that maintains the IC population, which biologically corresponds to normal tissue renewal. Drugs that non-selectively inhibit symmetric IC division would theoretically be similar to our present day pharmaceutical platform of chemotherapy agents, namely, they are designed to generically kill proliferating cells and carry

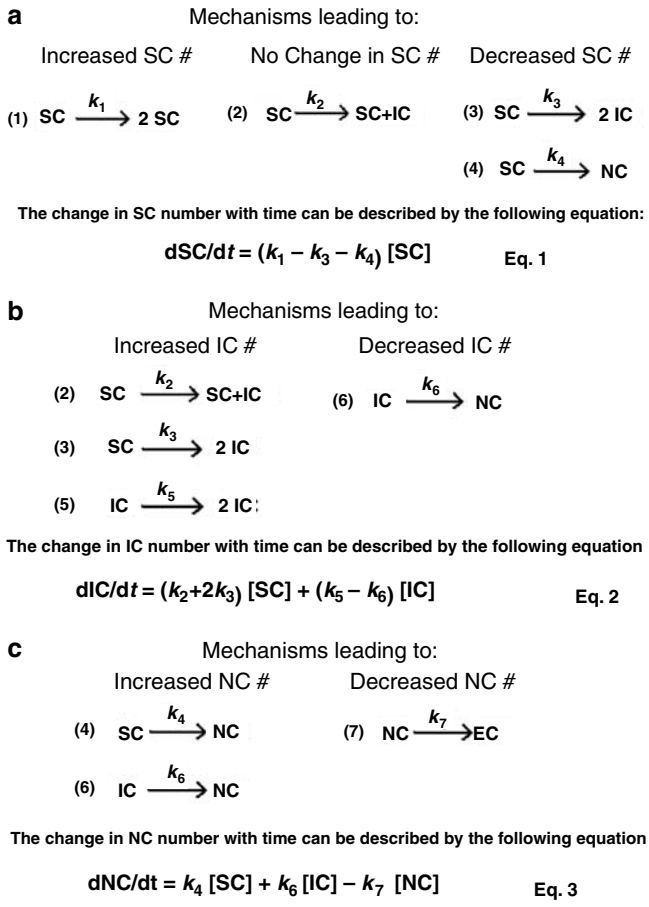

Figure 2 Kinetic relationships of cell populations. The mechanisms and equations in this Figure describe the kinetics for populations of (**a**) SCs, (**b**) ICs, (**c**) and NCs. The columns list reactions that lead to gain, no change, or decrease in cell numbers within the given population. The equation that describes the changes in SC, IC, or NC number with respect to time is given at the bottom of each figure.

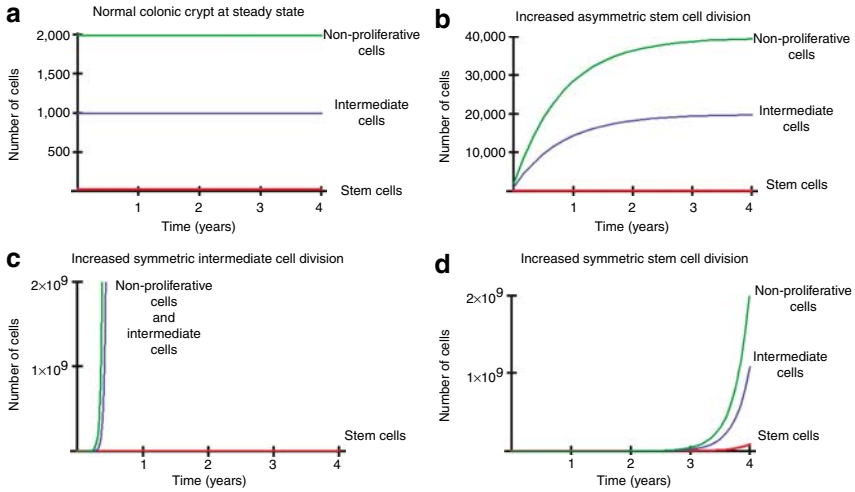

Figure 3 Model simulations. (**a**) Model output for normal colonic crypt populations showing a constant number of SC, IC, and NC with time, which is consistent with a steady-state condition in the colon crypt. (**b**) Output from increasing (20 ×) asymmetric SC division showing that the number of IC and NC asymptotically approaches a maximum, but SC does not increase. (**c**) Output from increasing (by as little as 1.2 ×) symmetric IC division and showing a rapid exponential increase in IC and NC, but not SC number. (**d**) Output from increasing (20 ×) symmetric SC division, showing a slow exponential increase in IC, NC, and SC. In this case, SCs represent a small subpopulation (~3%) of the total cell population. Note each relative time unit of computer output is equal to ~1 h (X axis) and thus 8,760 time units = ~1 year.

significant toxicity due to adverse effects on proliferating populations in normal tissues.

Another implication is that it will be necessary to identify which pathways that normally regulate symmetric SC division are altered in cancer SCs compared to normal cells. This is because any potential drug that does not selectively inhibit symmetric SC division in cancer tissues will have negative effects on symmetric SC division in normal tissues. Indiscriminately inhibiting symmetric SC division would also carry significant potential toxicity to normal tissues because our modeling indicates that this type of SC division is maintaining the SC population by counterbalancing SC loss. This could have adverse consequences due to toxicity from inhibiting normal tissue replacement (*e.g.*, via crypt fission) and repair of injured tissue.

Thus, the promise and challenge of the future for new and potentially curative cancer therapies lies in research on normal and cancer SCs. Currently, although the field is making advances at a relatively fast pace, it is still in its early stages of discovery. For instance, current studies on cancer SCs are mainly investigating the same pathways (*e.g.*, wnt, hedgehog, notch, PTEN, etc) to identify their roles in the different tumor types.[22,23] Given that the SCs in each tissue carry the genetic and cellular program that is responsible for the distinct organization and function of each tissue, it will be necessary to eventually determine which pathways are different in SCs in different tissues. Another challenge in developing drugs that selectively target symmetric SC division is that we will need specific SC markers to identify and isolate SCs, ways to measure the effect of agents on symmetric SC division, and methods to measure response of potential drugs on SCs *in vitro* and *in vivo*. This latter point will be challenging because cancer SCs are few in number and they are slowly proliferating so tumor responses may be slow and current methods of measuring antitumor responses will probably not apply to assessing the effect from an anti-SC drug. Nonetheless, although there is much work to be done in this field, cancer research appears to now have finally taken the first step in the right direction toward developing new anticancer therapies that have the potential to cure cancer patients.

METHODS

Kinetic relationships of cell populations. The kinetic relationships of cell populations in our model were based on biological evidence.[22,23] *SC population*: Reactions shown in **Figure 2a** indicate that the number of SC will increase by symmetric SC division, SC→2 SCs (Rxn 1). The number of SC will decrease by processes described by SC→2 ICs (Rxn 3) and SC→NC (Rxn 4). In asymmetric SC division, SC→SC + IC (Rxn 2), the number of SCs does not change. Based on this logic, the change in SC number with time is described by Eq. 1, which accounts for both SC production and SC loss.

IC population: Overall, the number of ICs (**Figure 2b**) is increased by another type of symmetric SC division, SC→2 ICs (Rxn 3), asymmetric SC division, SC→SC + IC (Rxn 2), and by symmetric IC division, IC→2 ICs (Rxn 5). The number of ICs will be decreased by IC growth termination IC→NC (Rxn 6). The change in IC number with time is described by Eq. 2, which accounts for both IC production and IC loss. As, by definition, the IC population is normally not self-renewing, $k_6 > k_5$ in Eq. 2.

NC population: Overall, the number of NC (**Figure 2c**) will be increased by termination of SC growth, SC→NC (Rxn 4) and termination of IC growth, IC→NC (Rxn 6). The number of NC will be decreased by eradication of NC from the system, NC→eradicated cell (Rxn 7). The change in NC number with time is described by Eq. 3, which accounts for both NC production and NC loss.

Kinetic relationships of cell populations in tissues. *Normal tissues*: In healthy normal tissues, cell populations are in steady state, *i.e.*, the

number of cells produced is balanced by the number lost. Thus, change in the number of cells in proliferative (SC and IC), NC and total cell populations will be 0. The steady-state conditions expressed in equation form are:

$$\text{For the SC population}: k_1 = k_3 + k_4 \qquad \text{(Eq. 4)}$$

$$\text{For the IC population}: (k_2 + 2k_3)[\text{SC}] = (k_6 - k_5)[\text{IC}] \qquad \text{(Eq. 5)}$$

$$\text{For the NC population}: k_4[\text{SC}] + k_6[\text{IC}] = (k_7)[\text{NC}] \qquad \text{(Eq. 6)}$$

Each equation above accounts for the balance, at steady state, between cell production (left side of equation), and loss (right side of equation).

Malignant tissues: In cancers, cell populations are not in steady state, cell production is greater than cell loss, and SC, IC, and NC increase with time. Thus, for the malignant tissue case, the left side (expression for cell production) of each equation (Equations 4–6) is greater than the right side (cell loss).

Characteristics of colon cell populations. Like the cell population of the normal human colonic crypt, the normal cell population in our model contains ~3,000 cells. Of these, 1,000 are ICs, 2,000 are NCs, and 20 (~1%) are SCs. The model crypt renews every 5 days and the average cell cycle time is 30 h. In the model, colon cancer arises from changes in the normal cell population. Simulated cancers, like biologic CRCs, typically contain 10^8–10^{13} cells. In the model, tumors contain SC, IC, and NC. The simulated cancer has an SC:total cell ratio (*i.e.*, SC/(SC + IC + NC)) that is approximately equal to the same ratio in the normal model cell (20:3,020).

Rate constant values. *Symmetric IC division*: The rate constant value for symmetric division of IC was estimated from the biologic cell cycle time of human colonocytes (average of 30 h).[24] Using this average cell cycle time and according to standard rate equation calculations,[25] the rate constant k_5 for symmetric IC division is estimated to be 0.023/h.

Asymmetric SC division: The rate constant value for asymmetric division of SC was estimated from the cell cycle time (100 h) of SCs at the bottom of the normal crypt (Bach *et al.*,[26] BMB, and OAR unpublished data). Using this SC cycle time and standard rate equations, the rate constant k_2 for SC asymmetric IV division is estimated to be 0.00693/h.

Symmetric SC division: The rate constant value for symmetric division of SC was estimated from biologic information indicating that only one out of every 300 crypts undergoes crypt fission at any given time and that crypt fission is a process that results from symmetric SC division.[27–30] Thus, in the model, symmetric SC division is 300 times slower than asymmetric SC division and k_1 is estimated to be 0.000023. In contrast to the normal biological colon, in neoplastic colonic epithelium of familial adenomatous polyposis patients, one in 15 crypts undergoes crypt fission at any given time.[29] Thus, in modeling cancer we assumed that k_1 for symmetrical SC division is 0.0046/h. Values for other rate constants were set based on the expressions in Eq. 4–6 and that the IC population is by definition not self renewing under normal conditions (*i.e.*, $k_6 > k_5$).

Computer modeling. Rate equations were written and then solved using Mathematica equation-solving software (version 5.2, Wolfram Research, Champaign, IL). The estimated rate constant values were used for modeling steady state conditions in normal colonic crypts (Methods) and then the rate constants were individually increased or decreased to study effects of perturbations on different cell populations. Model output was graphically displayed as number of SC, IC, and NC cells (Y axis) versus time (X axis). As rate constant values are expressed per hour, each relative time unit of computer output is equal to ~1 h and 8,760 output units = 1 year.

ACKNOWLEDGMENTS

This study was supported in part by the generous financial support of the Will and Jeanne Caldwell Fund for Cancer Research.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

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